Overexpression of EP300-interacting inhibitor of differentiation 3 predicts poor prognosis in patients with glioblastoma multiforme

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Abstract: EP300-interacting inhibitor of differentiation 3 (EID3) is a member of the IED family and has been associated with tumorigenesis and tumor development in different cancer types. However, the role of EID3 in glioblastoma multiforme (GBM) prognosis is not clear. Whole transcriptome sequencing data of 249 and 149 GBM patients were collected from the Chinese Glioma Genome Atlas (CGGA) and The Cancer Genome Atlas (TCGA) database respectively. The correlation between EID3 expression and overall survival (OS)/clinical pathologic features of GBM patients was investigated. Based on the Wilcoxon rank-sum test, EID3 expression in GBM tissues was significantly lower than in normal brain tissues (P < 0.001), and significantly higher than in LGG (low-grade glioma) (P < 0.001). There was a significant correlation between high EID3 expression with poor OS in CGGA (P = 0.049) and TCGA data (P = 0.024). Gene set enrichment analysis (GSEA) data analysis revealed a significant difference (FDR < 0.25, NOM p-value < 0.05) in the enrichment of MSigDB Collection (h.all.v6.2.symbols.gmt). A total of eight enriched pathways were identified in the high EID3 expression group, including Myc Targets V1, Kras signaling DN, and DNA repair pathways. Multivariate Cox regression analysis indicated that high expression of EID3 correlated with poor OS (P = 0.032, HR = 1.41, CI: 1.03-1.90). We conclude that EID3 could serve as an independent factor for predicting the prognosis of patients with GBM. Moreover, it is associated with GBM development through the regulation of the Myc Targets, Kras signaling DN, and DNA repair pathways.

Keywords: EID3, prognosis, glioblastoma, overall survival

Introduction

Brain tumors are a collection of abnormal cells in the brain and they can either be cancerous or benign tumors. Cancerous tumors can be highly invasive and can cause fatal tumor diseases [1]. Gliomas are tumors that start in the glial cells and are the most common malignant primary brain tumors in adults accounting for 50% of primary intracranial tumors [2]. According to the World Health Organization (WHO) classification of Central Nervous System Tumors, diffuse gliomas are graded from I to IV [3]. Lower-grade gliomas (WHO grades I and II) and high-grade gliomas (grade III and IV) differ in terms of their histopathological appearance and molecular parameters. The most malignant glioblastoma multiforme is grade IV since it is the most aggressive and infiltrative. GBM tumors show pleomorphic characteristics, while some of the histological features of are poor differentiation pathological mitosis, microvascular proliferation and neoplastic necrosis [4]. GBM is highly resistant to radiotherapy and chemotherapy and this has been associated with poor prognosis in GBM patients [5, 6]. Therefore, there is a need for extensive research to find better treatment modalities for GBM.

Current standard therapies for glioblastoma include surgical resection, radiotherapy, and adjuvant temozolomide (TMZ) chemotherapy [7-9]. However, the capacity of GBM to aggres-
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EP300-interacting inhibitor of differentiation 3 (EID3) is a member of the EID family which inhibits cellular differentiation by binding to CREB-binding protein/p300 and suppressing transcription [13]. EID3 is highly expressed in human testis [14]. EID3 is a p300 acetyltransferase inhibitor which can directly regulate DNA methyltransferase 3A (DNMT3A) transcription and differentiation into neural stem-like cells (uNSCL) in the umbilical cord mesenchymal stem cells (UMSC) [15]. Furthermore, it has been reported that EID3 positively regulates the proliferation of colorectal cancer cells and this is associated with poorer prognosis [16]. Previous studies have reported that patients with colorectal cancer (CRC) have significantly hypermethylated EID3, hence EID3 is considered as a potential methylation marker in colon cancer [17]. However, EID3 expression as a predictor of glioblastoma prognosis has not been well established. Therefore, this study investigated the clinical relevance of EID3 expression in the prognosis of GBM patients based on CGGA and TCGA data.

Materials and methods

Data collection

Transcriptome sequencing data and clinical information of 249 glioblastoma patients and 188 low-grade gliomas were retrieved from the Chinese Glioma Genome Atlas (CGGA, http://www.CGGA.org.cn) and the connected RNA-seq data and clinical information extracted. Clinical data collected included the Overall survival (OS), which was calculated from the date of diagnosing to death or date of the last follow-up. mRNA expression of EID3 in glioblastoma was analyzed and expression values not available in the dataset were excluded. Another 159 glioblastoma and 510 low-grade glioma patient’s transcriptome sequencing data and clinical information data were retrieved from The Cancer Genome Atlas (TCGA) database (http://cancergenome.ucsf.edu). EID3 expression data of 1152 normal brain tissues were downloaded from The Genotype-Tissue Expression (GTEx) database (https://gtexportal.org/home/index.html).

Statistical analysis

The overall survival (OS) was defined as the date of disease diagnosing to the death of the patient or date of the last follow-up. Kaplan-Meier survival curve and log-rank test were used to estimate the correlation between EID3 expression and OS. Wilcoxon rank-sum test or Kruskal-Wallis test estimated the correlation between clinical pathologic features and EID3 expression. R software (https://www.r-project.org/, v3.4.3) was used for statistical analysis. P-value < 0.05 indicated significance.

Gene set enrichment analysis (GSEA)

GSEA was used to analyze the difference in overall survival between high and low EID3 expression groups. GSEA verifies whether there exists a statistically significant difference between two biological states in a pre-defined set of genes [18]. For gene sampling approach, 1000 permutations were used. The different expression levels of EID3 were considered as phenotype label. The nominal P-value, as well as normalized enrichment score (NES), were used in data processing during the sorting of the pathways enriched in each phenotype.

Results

High expression of EID3 is associated with poor prognosis of GBM patients

Table 1 presents the patients’ clinical information such as sex, age, recurrence, IDH mutation and 1p19q codeletion status. A total of 249 patients (102 female and 147 male) in CGGA
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database were included in this study, out of which 49.5% (n = 123) of the patients were below 50 years old, 76.7% (n = 191) were IDH wildtype, 82.3% (n = 205) contained 1p19q non-code, 56.2% (n = 140) were newly diagnosed, while 43.8% (n = 109) of patients were diagnosed as recurrent GBM.

The median follow-up time of the 249 GBM patients obtained from CGGA data and analyzed by K-M survival analysis was 12 months. Patients with high EID3 expression had a poor OS (P = 0.049, Figure 1A). TCGA data confirmed the association between high EID3 expression and GBM prognosis and the results revealed that poor OS was significantly associated with high EID3 expression of GBM patients (P = 0.024) (Figure 1D).

**EID3 is independently correlated with prognosis of glioblastoma**

**EID3-related signaling pathways**

GSEA analysis showed significant difference (FDR < 0.25, NOM p-value < 0.05) in the enrichment of MSigDB Collection (h.all.v6.2.symbols.gmt). In addition, a total of eight pathways were shown to be differently enriched with high EID3 expression, including Myc Targets V1, Kras signaling DN, DNA Repair, UV response DN pathway, inflammatory response, TNFA signaling through NFKB, epithelial-mesenchymal transition and MYC targets V2 (Figure 4A-H; Table 3). This revealed the key role of EID3 in the progression of glioblastoma.

**Discussion**

This study confirmed that overexpression of EID3 is correlated with poor overall survival of glioblastoma patients. Univariate and multivariate Cox regression analysis revealed that EID3 expression can be used as a prognostic predictor for glioblastoma patients. Moreover, overexpression of EID3 in glioblastoma tissues was positively correlated with IDH wildtype and 1p19q non-code status. GSEA analysis also showed that overexpression of EID3

**Table 1. Characteristics of patients with GBM based on CGGA**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number of cases</th>
<th>Percentages (%)</th>
</tr>
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<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 50</td>
<td>123</td>
<td>49.4</td>
</tr>
<tr>
<td>≥ 50</td>
<td>126</td>
<td>50.6</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>147</td>
<td>59.0</td>
</tr>
<tr>
<td>Female</td>
<td>102</td>
<td>41.0</td>
</tr>
<tr>
<td>Primary or Recurrent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>140</td>
<td>56.2</td>
</tr>
<tr>
<td>Recurrent</td>
<td>109</td>
<td>43.8</td>
</tr>
<tr>
<td>IDH mutation status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wildtype</td>
<td>191</td>
<td>76.7</td>
</tr>
<tr>
<td>Mutant</td>
<td>48</td>
<td>19.3</td>
</tr>
<tr>
<td>Not Available</td>
<td>10</td>
<td>4.0</td>
</tr>
<tr>
<td>1p19q codeletion status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non codel</td>
<td>205</td>
<td>82.3</td>
</tr>
<tr>
<td>Codel</td>
<td>13</td>
<td>5.2</td>
</tr>
<tr>
<td>Not Available</td>
<td>31</td>
<td>12.4</td>
</tr>
</tbody>
</table>

Wilcoxon rank-sum test compared the expression of EID3 in 249 glioblastoma and 188 low-grade glioma tissues from CGGA data, 159 glioblastoma and 510 low-grade gliomas tissues from TCGA data and 1152 normal brain tissues from the GTEx database. The results revealed that EID3 expression in GBM tissues was significantly lower than that in normal brain tissues (P < 0.001) (Figure 2A, 2B) but significantly higher than that in LGG (P < 0.001) (Figure 2C, 2D). Correlation analysis of EID3 expression with clinical features in 249 samples revealed that EID3 expression was not related to gender, or primary (newly diagnosed) and recurrent (diagnosed as recurrent) status (Figure 3A, 3B). Further, the results showed that increased EID3 expression was positively associated with IDH1 mutation (P < 0.001) (Figure 3C) and 1q/19p co-deletion status (P < 0.001) (Figure 3D).
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Figure 1. High expression of EID3 is associated with poor OS in patients with glioblastoma. (A-C from CGGA data) (A) Kaplan-Meier curves, (B) Number at risk, (C) Number of censoring of OS in GBM. (D-F from TCGA data) (D) Kaplan-Meier curves, (E) Number at risk, (F) Number of censoring of OS in GBM. OS, over survival.
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Figure 2. Wilcoxon rank-sum test analysis. A. EID3 expression in GBM (glioblastoma multiforme) tissue of CGGA data and normal brain tissues of GTEx database (P < 0.001). B. EID3 expression in GBM (glioblastoma multiforme) tissue of TCGA data and normal brain tissues of GTEx database (P < 0.001). C. EID3 expression in GBM tissue and LGG (low-grade glioma) tissue of CGGA database (P < 0.001). D. EID3 expression in GBM tissue and LGG (low-grade glioma) tissue of the TCGA database (P < 0.001).

was associated with the Myc Targets pathway, the Kras signaling DN pathway, the DNA repair pathway, the UV response DN pathway, the inflammatory response pathway, the TNFA signaling through the NFKB pathway, and the epithelial-mesenchymal transition pathway.

The role of EID3 in tumor prognosis is not clear. However, previous studies have shown that overexpression of EID3 is associated with poor prognosis, promotion of cell proliferation, and induced therapeutic drug resistance in colorectal cancer. A decrease in the expression of EID3, is associated with a reduction in colorectal cancer cells proliferation and therapeutic resistance [16]. Hassan Ashktorab performed DNA methylation analysis of tumor tissues from African American colorectal cancer patients and identifies EID3 as a new methylation prognostic maker [17]. Koji Hontani showed that high levels of serum anti-EID3 antibodies are an indicator of a high risk of tumor recurrence in patients with non-functional pancreatic neuroendocrine tumors [19]. Our study revealed that overexpression of EID3 is also associated with poor survival of glioblastoma patients, suggesting that EID3 expression can be used as a prognostic predictor for glioblastoma patients.

GSEA analysis showed that EID3 is involved in the Myc targets pathway, the Kras signaling DN pathway, the DNA repair pathway, the UV response DN pathway, the inflammatory response pathway, the TNFA signaling through NFKB pathway, and the epithelial-mesenchymal transition pathway. EID3 can bind to CBP/p300 which is one of the important molecules of the MYC pathway [14, 20]. Myc which is a critical transcription factor, is reported to have a number of carcinogenic abilities, such as tumor cell proliferation, growth, angiogenesis,
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and genomic instability [21]. In GBM, dysregulation of the Myc gene has been reported to cause an elevation of c-Myc expression and tumor progression. c-Myc maintains glioma stem cells self-renewal and tumorigenic potential [22-27]. Oncogenic KRAS activates the RAS/MAPK pathway and promotes GBM cell proliferation and growth. EDI3 is associated with the Kras signaling by the DN pathway, hence it has a tumor-suppressing effect [23]. Glioblastoma can resist DNA damage induced by chemotherapeutic drugs through the activation of the DNA repair pathway. EDI3 is associated with the sensitivity of cancer cells to

Figure 3. Association of EID3 expression and clinicopathologic characteristics. (A) Gender, (B) Primary or recurrent, (C) IDH mutation status, (D) 1p19q codeletion status.

Table 2. Univariate and multivariate regression survival model of prognostic covariates in GBM patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Hazard ratio</th>
<th>CI 95</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Univariate analysis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years) (≥ 50 vs. &lt; 50)</td>
<td>1.13</td>
<td>0.83-1.53</td>
<td>0.43</td>
</tr>
<tr>
<td>Gender (Female vs. Male)</td>
<td>1.02</td>
<td>0.75-1.39</td>
<td>0.898</td>
</tr>
<tr>
<td>Primary or Recurrent (Recurrent vs. Primary)</td>
<td>1.72</td>
<td>1.26-2.36</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>IDH mutation status (Mutant vs. Wildtype)</td>
<td>0.47</td>
<td>0.30-0.74</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>1p19q codeletion status (Code vs. Non-codel)</td>
<td>1.89</td>
<td>0.83-4.28</td>
<td>0.127</td>
</tr>
<tr>
<td>EID3</td>
<td>1.22</td>
<td>1.10-1.35</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>Multivariate analysis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years) (≥ 50 vs. &lt; 50)</td>
<td>1.14</td>
<td>0.80-1.60</td>
<td>0.473</td>
</tr>
<tr>
<td>Gender (Female vs. Male)</td>
<td>1.02</td>
<td>0.73-1.4</td>
<td>0.892</td>
</tr>
<tr>
<td>Primary or Recurrent (Recurrent vs. Primary)</td>
<td>2.06</td>
<td>1.43-3.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>IDH mutation status (Mutant vs. Wildtype)</td>
<td>0.65</td>
<td>0.37-1.10</td>
<td>0.115</td>
</tr>
<tr>
<td>1p19q codeletion status (Code vs. Non-codel)</td>
<td>1.35</td>
<td>0.56-3.30</td>
<td>0.505</td>
</tr>
<tr>
<td>EID3</td>
<td>1.41</td>
<td>1.03-1.90</td>
<td>0.032</td>
</tr>
</tbody>
</table>
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A. HALLMARK_KRAS_SIGNALING_DN
   p-value 0.0043
   p.adjust 0.044

B. HALLMARK_TNFA_SIGNALING_VIA_NFKB
   p-value 0.0064
   p.adjust 0.044

C. HALLMARK_INFLAMMATORY_RESPONSE
   p-value 0.0057
   p.adjust 0.044

D. HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION
   p-value 0.007
   p.adjust 0.044

E. HALLMARK_UV_RESPONSE_DN
   p-value 0.0055
   p.adjust 0.044

F. HALLMARK_MYC_TARGETS_V1
   p-value 0.0012
   p.adjust 0.044

G. HALLMARK_DNA_REPAIR
   p-value 0.0049
   p.adjust 0.044

H. HALLMARK_MYC_TARGETS_V2
   p-value 0.044
   p.adjust 0.0668
DNA damaging agents [28, 29]. EID protein through the ubiquitin-dependent proteolysis is rapidly degraded by binding to the retinoblastoma tumor suppressor protein (pRB) in the proteasome at the end of the cell cycle [30, 31]. However, it cannot be combined with EID due to inactivation of its functions by pRB mutations. This inhibits EID degradation and cell differentiation. Therefore, EID family members promote tumorigenesis by inhibiting cell differentiation [13, 14, 32]. EID3 not only has methylation, but also demethylation ability [15]. This research provides a deeper understanding of the relationship between EID3 expression and glioblastoma. However, this study did not investigate the relationship between EID3 mRNA expression and EID3 protein expression, which should be considered in future studies.

Conclusions

This study demonstrates that EID3 expression can be used as a prognostic marker of glioblastoma patients. EID3 is involved in GBM development through the Myc targets pathway, the Kras signaling DN pathway, and the DNA repair pathway. However, future studies should examine the biological role of EID3 in GBM.

Acknowledgements

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Disclosure of conflict of interest

None.

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